

PATENT COOPERATION TREATY

RECEIVED

11 OCT 2004

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

Rec'd PCT/PTO

27 JAN 2005

PCT

To:

FRANKS, Barry
Amersham plc
Amersham Place
Little Chalfont
Buckinghamshire HP7 9NA
GRANDE BRETAGNE

DL	—
FORMALITIES	HSL ✓
PAI OFF	MRB
ON DE	—
CASE NO:	PA0255-

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

07.10.2004

Applicant's or agent's file reference
PA0255-PCT

IMPORTANT NOTIFICATION

International application No.
PCT/GB 03/03190

International filing date (day/month/year)
28.07.2003

Priority date (day/month/year)
30.07.2002

Applicant
AMERSHAM BIOSCIENCES UK LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Evers, A

Tel. +49 89 2399-7061



BEST AVAILABLE COPY

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)



REC'D 11 OCT 2004

WIPO PCT

Rec'd PCT/PTO 27 JAN 2005

Applicant's or agent's file reference PA0255-PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/GB 03/03190	International filing date (day/month/year) 28.07.2003	Priority date (day/month/year) 30.07.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/58		
Applicant AMERSHAM BIOSCIENCES UK LIMITED et al.		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of 8 sheets, including this cover sheet.
	<input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of 6 sheets.
3.	This report contains indications relating to the following items: <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the opinion II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application

Date of submission of the demand 04.02.2004	Date of completion of this report 07.10.2004
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Fausti, S Telephone No. +49 89 2399-7389 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB 03/03190

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*):

Description, Pages

1-19 as originally filed

Claims, Numbers

1-19 received on 26.07.2004 with letter of 23.07.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB 03/03190

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-19
	No: Claims	-
Inventive step (IS)	Yes: Claims	-
	No: Claims	1-19
Industrial applicability (IA)	Yes: Claims	1-19
	No: Claims	-

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/03190

A. AMENDMENTS (Art. 34(2)(b) PCT).

- A.1 Independent claim 1 results from the combination of original claims 1 and 2. The other claims have been re-numbered accordingly. The amendments meet therefore the requirements of Art. 34(2)(b) PCT.
- A.2 By means of these amendments, the scope of the claims has been effectively restricted to the searched subject-matter (see Box I.2 of the International Search Report). Hence, a complete examination of the claimed subject-matter is possible and has been carried out.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. DOCUMENTS.

Reference is made to the following documents:

- D1: US 3835139;
D2: Faller T. et Al., *Chemical Communications* (1997) vol. 16, pages 1529-1530;
D3: Schuler B. et Al., *Bioconjugate Chemistry* (2002) vol. 13, no. 5, pages 1039-1043;
D4: WO 02/099424 A;
D5: WO 02/099432 A;
D6: Klein G. et Al., *Chemical Communications* (2001) vol. 7, no. 6, pages 561-562;
D7: EP 0857721 A .

- 1.1 D1 discloses the methyl 7-(dimethyl-carbamoyl-thio)-acridone-2-carboxylate as intermediate in the synthesis of acridone derivatives against allergy (see example 13).
- 1.2 D2 discloses acridone derivatives for the purpose of labelling polypeptides with fluorescent dyes (see abstract and page 1529, left-hand column, last paragraph). In particular, the acridone dye is derivatized at the nitrogen atom with an activated carboxylic group, i.e. succinimide ester, which is highly reactive towards the

peptide primary amino groups (see compound 1 on page 1529, left-hand column).

- 1.3 D3 discloses a method for site-specific labelling of polypeptides with fluorescent dyes (see abstract). According to this method, a dye derivative comprising a thioester group reacts with the peptide N-terminal Cysteine like in the native chemical ligation reaction (see also scheme 2).
- 1.3^a In the specific embodiment, a cyanine dye is used, but it is suggested that the method could be applied to any labelling agent, for which an activated succinimide derivative is available (see abstract). Starting from succinimide derivative, the reactive thioester group for peptide ligation is attached to the dye (see scheme 1 and page 1039, right-hand column, first paragraph).
- 1.4 D4 and D5 disclose acridone and quinacridone derivatives comprising reactive groups ("target bonding groups") for labelling target materials like proteins and peptides (see: D4, claims 13, 27-29; D5, claims 11, 27-29). Example of reactive groups are carboxyl, succinimidyl ester, isothiocyanate, maleimide, acid halide, hydrazide, etc... (see: D4, claim 7; D5, claim 6).
- 1.6 D6 discloses a quinacridone derivative having ethylenediamino groups bound to the nitrogen atoms for metal ion detection (see scheme 1).
- 1.7 D7 discloses acridone derivatives for the treatment of asthma, allergies, etc (see abstract). Some of these derivatives and the synthetic intermediate compounds consist of a substituted acridone nucleus, which bear various functional groups like ester, primary amino, secondary amino and halo groups (see for example table 5, example 1 and production example 9).
2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).
 - 2.1 Claims 1-19 relates to compounds and methods for labelling proteins of biological interests. These compounds and methods can be made and applied in industrial activities for the production of chemical reagents, for analytical purposes and/or for protein purification, hence they are to be considered industrially applicable according to article 33(4) PCT.
3. NOVELTY (Art. 33(2) PCT).

- 3.1 The subject-matter of claims 1-19 is novel over the available prior art as explained below.
- 3.2 The compound of formula (I), as defined in independent claims 1 and 14, is novel over the acridone-2-carboxylate intermediate disclosed in D1 in view of the linking moiety "L¹-M", which is present in the claimed compound and comprises at least one carbon atom. Such a linking moiety is absent in the acridone intermediate of D1, wherein the acridone moiety is directly bound to the carbonyl-thio functionality (see point 1.1 above).
- 3.2^a None of the compounds disclosed in D2, D3, D6 and D7 comprises all the technical features referred to in independent claims 1 and 14, i.e. an acridone or quinacridone dye and a targeting bonding group selected from a carboxylic acid thioester and a 1,2-aminothiol group (see points 1.2, 1.3, 1.6 and 1.7 above).
- 3.3 The subject-matter of dependent claims 2-13 and 15-19 is also novel for the same reasons.

4. INVENTIVE STEP (Art. 33(3) PCT).

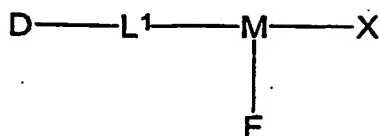
- 4.1 Document D2 is considered to represent the relevant state of the art because it discloses acridone derivatives for peptide labelling. The subject-matter of claims 1 and 14 differs in the thioester or the specific thioester-reactive functionality (i.e. the 1,2-aminothiol group) (see point 1.2 above).
- 4.1^a Irrespective of the fact that D2 specifically concerns labelling methods for detecting peptide fragments in mass spectrometry, D2 can be considered among the relevant prior art. The skilled person would have taken D2 into account in order to find a solution to the problem posed because there is no technical feature limiting the claimed subject-matter to a specific technical field of application.
- 4.2 In view of the disclosure of D2, the problem to be solved may be regarded as the provision of alternative reagents and methods for labelling peptides or proteins with acridone or quinacridone fluorescent dyes.
- 4.3 The solution proposed in independent claims 1 and 14, i.e. dye derivatives having a thioester or a thioester-reactive group for site-specific labelling, does not appear to involve any inventive step over D2, taken in combination with D3.

- 4.4 Labelling procedures for site-specific peptide labelling involving thioester functionalities are known from D3 (see for example point 1.3 above). According to these labelling procedures, a fluorescent label is attached to a protein or peptide by means of a reaction which is analogous to the native chemical ligation. The skilled person would therefore have applied such a procedure by introducing the thioester functionality in the acridone derivative of D2 in order to solve the problem posed, thereby obtaining labelling reagents and methods according to claims 1 and 14.
- 4.4^a In particular, D3 suggests that this labelling procedure can be applied to a wide range of fluorescent labels bearing a succinimide group, like the acridone derivative disclosed in D2, and explicitly indicates how to introduce the thioester functionality in place of the succinimide group (see point 1.3^a above).
- 4.4^b No unexpected effects and properties, which could not be foreseen in the light of the prior art, are apparently achieved by means of this labelling procedure, and therefore the claimed subject-matter is considered to lack an inventive step.
- 4.4^c The International Examination Authority is of the opinion that the observation made by the authors of D2 with respect to the improved fragment distribution (see D2, page 1530, lines 7-11) is irrelevant for the inventive step reasoning above and would not have prevented the skilled person to find the solution to the problem posed in this document. In the passage concerned, D2 simply indicates that an advantage for the mass spectrometric analysis is achieved by means of stable amide bonds between the peptidic analytes and the labelling agents because of a reduced number of basic sites in the analyte molecules. As the labelling procedure of D3 also involves basic groups of the polypeptide moiety (i.e. amines) and results in the formation of amide bonds, the same advantage could be achieved by means of the labelling procedure of D3.
- 4.5 Independently from the reasoning above, the claimed subject-matter does not involve any inventive step taking D3 as the closest prior art.
- 4.5^a D3 discloses the site-specific fluorescent labelling of peptides by means of a native chemical ligation strategy (see point 1.3 above). The subject-matter of claims 1 and 14 differs from the labelling reagent and the labelling method explicitly disclosed in D3 in that the chromophore is an acridone or quinacridone dye, rather than a cyanine dye (see point 1.3^a above).
- 4.6 The problem to be solved may therefore be regarded as the provision of alternative reagents and methods for the site-specific fluorescent labelling of peptides or proteins.

- 4.7 The solution proposed in claims 1 and 14, which consists in the choice of an acridone or quinacridone chromophore, cannot be considered as involving any inventive step because the authors of D3 indicate that their label ligation approach could be applied to a wide range of labelling moieties provided in activated succinimidyl ester forms (see again 1.3^a above).
- 4.7^a As the prior art discloses such an activated form of the acridone dye (see point 1.2 above), the skilled person would have considered to apply the labelling method of D3 with the activated acridone moiety of D2 in order to solve the problem posed, thereby obtaining a compound (labelling reagent) and a method as defined in claims 1 and 14. In view of the disclosure of D2 and the indication for broad applicability of the labelling method of D3, the claimed subject-matter is to be considered a selection among the equivalent alternatives coming within the skilled person knowledge. Such a selection, namely the choice of an acridone or quinacridone chromophore for the labelling method of D3, can only be regarded as inventive if it presents unexpected effects or properties. No such effects or properties are indicated in the application, and therefore the subject-matter of claims 1 and 14 lacks an inventive step.
- 4.8 Dependent claims 2-13 and 15-19 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, given the disclosure of the prior art.
- 4.8^a The incorporation of an affinity tag in the labelling agent for purification/isolation purposes comes within the scope of the customary practice followed by persons skilled in the art.
- 4.8^b As the idea of using native chemical ligation for labelling purposes is known (see for example point 1.3 above), the skilled person could also have considered to use labelled peptides and to attach them either to the N-terminus or to the C-terminus of the desired polypeptide by native chemical ligation.
- 4.8^c Quinacridone dyes are known (see for example point 1.6 above) and are structurally so similar to acridone dyes that the skilled person could have considered to substitute the fluorophore in the acridone derivatives of D2 with quinacridone.

Claims

1. A compound having the formula (I):



5

(I)

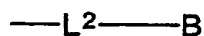
wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

- 10 M is a group adapted for attaching to F;

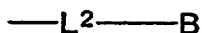
X is selected from hydrogen or the group:



wherein B is an affinity tag; and

- 15 L¹ and L² each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C₁ – C₄ alkyl.

2. A compound according to claim 1 wherein X is the group:



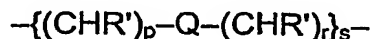
20

wherein B and L² are hereinbefore defined.

3. A compound according to claim 1 or claim 2 wherein each of L¹ and L² contains from 2 to 30 atoms.

25

4. A compound according to claim 1 or claim 2 wherein L¹ and L² are independently selected from the group:



ART 34 AMDT

AMENDED SHEET

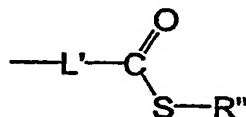
where Q is selected from: $-\text{CHR}'-$, $-\text{NR}'-$, $-\text{O}-$, $-\text{CH}=\text{CH}-$, $-\text{Ar}-$ and $-\text{CO}-\text{NH}-$; R' is hydrogen or $\text{C}_1 - \text{C}_4$ alkyl, p is 0 - 5, r is 1 - 5 and s is 1 or 2.

5. A compound according to claim 4 wherein Q is selected from $-\text{CHR}'-$, $-\text{O}-$ and $-\text{CO}-\text{NH}-$, where R' is hereinbefore defined.

6. A compound according to any of claims 1 to 5 wherein said affinity tag is selected from biotin and desthiobiotin.

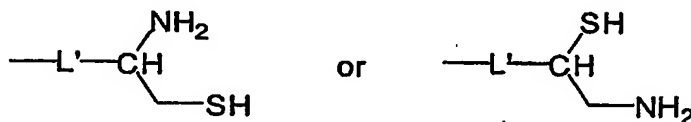
7. A compound according to any of claims 1 to 5 wherein said affinity tag is selected from his-tag, iminodiacetic acid and nitrilotriacetic acid.

8. A compound according to any of claims 1 to 7 wherein the target bonding group F is a carboxylic acid thioester of formula:



wherein L' is a bond or is a group containing from 1 - 30 linked atoms selected from carbon atoms and optionally one or more groups selected from $-\text{NH}-$, $-\text{O}-$ and $-\text{CO}-\text{NH}-$; and R'' is $\text{C}_1 - \text{C}_4$ alkyl, $\text{C}_6 - \text{C}_{10}$ aryl, or $\text{C}_7 - \text{C}_{15}$ aralkyl, which may be optionally substituted with sulphonate; or is the group $-(\text{CH}_2)_2-\text{CONH}_2$.

9. A compound according to any of claims 1 to 7 wherein the target bonding group F is a 1,2-aminothiol group of formula:

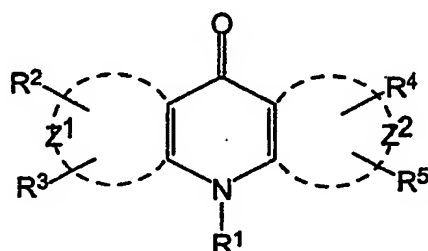


wherein L' is hereinbefore defined.

ART 34 AMDT

AMENDED SHEET

10. A compound according to any of claims 1 to 9 wherein the compound is an acridone dye having the formula (II):



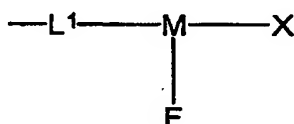
(II)

wherein:

groups R² and R³ are attached to the Z¹ ring structure and groups R⁴ and R⁵ are attached to the Z² ring structure;

Z¹ and Z² independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

at least one of groups R¹, R², R³, R⁴ and R⁵ is a group W having the formula:



where F, M, X and L¹ are hereinbefore defined;

when any of said groups R¹, R², R³, R⁴ and R⁵ is not said group W, said remaining groups R², R³, R⁴ and R⁵ are independently selected from hydrogen, halogen, amide, cyano, mono- or di-C₁ – C₄ alkyl-substituted amino, carbonyl, carboxyl, C₁ – C₆ alkoxy, acrylate, vinyl, styryl, aryl,

heteroaryl, C₁ – C₂₀ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary ammonium and the group -(CH₂)ₙ-Y and,

when group R¹ is not said group W, it is selected from hydrogen, C₁ – C₂₀ alkyl, aralkyl and the group -(CH₂)ₙ-Y; and

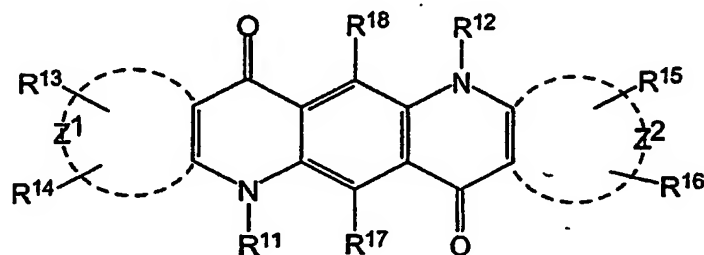
Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl; and n is an integer from 1 to 6;

ART 34 AMDT

AMENDED SHEET

provided that at least one of groups R^1 , R^2 , R^3 , R^4 and R^5 is a water solubilising group.

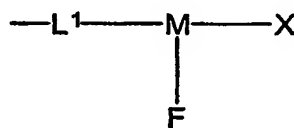
11. A compound according to any of claims 1 to 9 wherein the compound is a quinacridone dye having the formula (III):



(III)

wherein:

- groups R^{13} and R^{14} are attached to the Z^1 ring structure and groups R^{15} and R^{16} are attached to the Z^2 ring structure;
 Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;
 at least one of groups R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} is a group T having the formula:



where F, M, X and L^1 are hereinbefore defined;

- when any of said groups R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} is not said group T, said remaining groups R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} are independently selected from hydrogen, halogen, amide, cyano, mono- or di- $C_1 - C_4$ alkyl-substituted amino, carbonyl, carboxyl, $C_1 - C_6$ alkoxy, acrylate, vinyl, styryl, aryl, heteroaryl, $C_1 - C_{20}$ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary ammonium and the group $-(CH_2)_n-Y$; and,

ART 34 AMDT

AMENDED SHEET

when either of groups R^{11} and R^{12} is not said group T, it is selected from hydrogen, $C_1 - C_{20}$ alkyl, aralkyl and the group $-(CH_2)_n-Y$;

Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl; and n is an integer from 1 to 6;

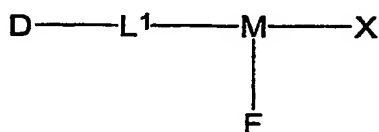
- 5 provided that at least one of groups R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} is a water solubilising group.

12. A compound according to claim 10 or claim 11 wherein Z^1 and Z^2 are selected independently from the group consisting of phenyl, pyridinyl,
10 naphthyl, quinolinyl and indolyl moieties.

13. A compound according to claim 10 or claim 11 wherein Z^1 and Z^2 are selected from phenyl and naphthyl moieties.

- 15 14. A method for labelling a protein of interest wherein said protein contains or is derivatised to contain an N-terminal cysteine, the method comprising:

- i) adding to a liquid containing said protein a compound of formula (I):



(I)

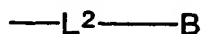
wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

- 25 M is a group adapted for attaching to F;

X is selected from hydrogen or the group:



where B is an affinity tag; and

- 30 L^1 and L^2 each independently comprise a group containing from 1 - 40 linked atoms selected from carbon atoms which may optionally include one or more

ART 34 AMDT

AMENDED SHEET

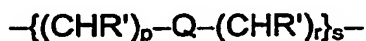
groups selected from $-NR'$ -, $-O$ -, $-CH=CH$ -, $-CO-NH$ - and phenylenyl groups, where R' is selected from hydrogen and $C_1 - C_4$ alkyl; and

ii) incubating said compound with said protein under conditions suitable for labelling said protein.

5

15. A compound according to claim 14 wherein each of L^1 and L^2 contains from 2 to 30 atoms.

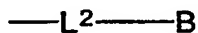
10 16. A method according to claim 14 wherein L^1 and L^2 are independently selected from the group:



15 where Q is selected from: $-CHR'$ -, $-NR'$ -, $-O$ -, $-CH=CH$ -, $-Ar$ - and $-CO-NH$ -; R' is hydrogen or $C_1 - C_4$ alkyl, p is 0 - 5, r is 1 - 5 and s is 1 or 2.

17. A method according to claim 16 wherein Q is selected from $-CHR'$ -, $-O$ - and $-CO-NH$ -, where R' is hereinbefore defined.

20 18. A method according to any of claims 14 to 17 wherein X is the group:



wherein B and L^2 are hereinbefore defined, said method further comprising separating and/or purifying the dye-labelled protein of interest by affinity chromatography.

25

19. A method according to any of claims 14 to 18 wherein said protein of interest is selected from antibody, antigen, protein, peptide, microbial materials, cells and cell membranes.

30

ART 34 AMDT

AMENDED SHEET

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.